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Formulation Selection, and Investigation of Azadirachtin-A Persistence in Some Terrestrial and Aquatic Components of a Forest Environment

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Abstract: Five commercial formulations of azadirachtin-A (AZ-A) Margosan-O®, Azatin-EC®, Neem-EC®, RH-9999 and Neemix® 4.5, were investigated for their volatilization and washoff potential in laboratory studies. Prior to the investigation, RH-9999 (a wettable powder) was mixed with water to provide an end-use formulation containing 35·6 g AZ-A kg^{-1}, while the remaining four formulations were investigated without dilution. Volatilization and washoff of AZ-A occurred more from white spruce foliage than from wax-coated glass plates. Neem-EC provided the lowest amount of loss, whereas Margosan-O provided the highest. Physical properties and atomization behaviour of the five formulations indicated that Azatin-EC was highly viscous and caused phase separation in droplets collected on glass plates after atomization in a rotary atomizer. RH-9999, despite its low viscosity, caused phase separation in droplets because of the heterogeneity of the wettable powder formulation. Based on the minimum loss of AZ-A due to volatilization and washoff from spruce foliage, and on the minimum potential for phase separation in droplets after atomization in a rotary atomizer, Neem-EC was considered to be the most appropriate choice for use in field studies to investigate environmental persistence and fate of AZ-A in terrestrial and aquatic matrices of a forest ecosystem.

The Neem-EC formulation was sprayed at 40 and 80 g AI ha^{-1} over single spruce trees and on litter and soil plots selected in a mixed-wood boreal forest in Ontario, Canada. In addition, outdoor aquaria containing stream water and sediment were also fortified with the formulation at 400 and 800 g AI ha^{-1}. Persistence of AZ-A was evaluated using one-year-old spruce needles, current-year shoots, spruce bark, litter, soil, stream water and sediment. The duration of persistence varied from 3 to 6 days in terrestrial matrices, whereas it ranged from 8 to 13 days in water, and 2 to 3 days in sediment. The half-life (DT_{50}) values ranged from 10·7 h (for soil) to 71·6 h (for spruce bark) at the lower dosage rate, and from 18·8 h (for litter) to 76·2 h (for bark) at the higher dosage rate. The DT_{50} value for stream water was about 35 h regardless of the dosage rate applied. The data indicated that AZ-A was appreciably labile and short-lived in different forestry matrices, with low DT_{50} values.

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1 INTRODUCTION

Awareness of the possible adverse side effects of the use of conventional neurotoxic pesticides in forest pest management programs[1] provided an impetus to look for alternatives. Among the various possible compounds, plant-derived natural products as insecticides have attracted attention in recent years.[2] Naturally occurring insecticides tend to be readily biodegradable,[3] and could be environmentally more acceptable than the synthetics.[4] One of the botanical products is the neem seed extract obtained from the tropical neem tree, *Azadirachta indica* A. Juss.[5] The extract contains a complex array of compounds which can cause a number of biological effects (i.e. anti-feedant, growth retardant, repeller, etc.) against a wide range of insects, including the destructive defoliators of forest trees.[6,7] The insecticidal properties of the extract are due to an isomeric mixture of tetranortriterpenoids collectively known as azadirachtins, and particularly due to the major isomer, azadirachtin-A (AZ-A; $C_{35}H_{44}O_{16}$), in the mixture.[8] The molecular structure, and some physical and toxicological properties of AZ-A are listed in the Pesticide Manual.[9] Currently, several neem formulations containing AZ-A are available for commercial[10] and experimental uses.[11] The material has a low toxicity to nontarget biota,[12,13] and is readily degradable in the environment.[14] As a result, the potential for adverse side effects is likely to be low for AZ-A compared to that of synthetic pesticides.[15]

At present, information is sparse on the behaviour, persistence and fate of neem-based AZ-A (referred to as the active ingredient, AI) in forestry matrices under field conditions, although some laboratory microcosm studies indicated high photo-instability and short duration of persistence in forestry foliage.[14] For acceptable insect control, the sprayed insecticide should persist in the target foliage in an active form during the critical period of insect development, and be at a concentration at or above the threshold level needed to cause mortality. Thus, optimum persistence in the target foliage is necessary for effective control of the insect pests. The data generated in the laboratory microcosm study[14] lacked environmental realism, because field persistence would vary depending upon the type of substrate, characteristics of the environment and prevailing climatic conditions.[16] If a neem-based formulation of AZ-A is to be registered in Canada for forestry use, then sufficient data are required on its behaviour under realistic field conditions, as stipulated in the Pest Control Products Act (PCPA) of Canada.

The objectives of the present study were (i) to determine the loss of AZ-A by volatilization and washoff from glass slides and white spruce foliage after treatment with five commercial formulations, (ii) to investigate the physical properties and behaviour of the formulations in rotary and pressure atomizers, (iii) to determine the most appropriate formulation that would minimize phase separation in droplets during atomization, and maximize retention of AZ-A in target foliage after volatilization and washoff, and (iv) to use the selected formulation under field conditions to investigate spray deposition, persistence and dissipation patterns of AZ-A in various forestry matrices in the real-world situation.

2 MATERIALS AND METHODS

The investigation consisted of two parts. Part I comprised a laboratory study to determine the volatilization and wash-off of AZ-A from treated surfaces, to investigate the physical properties [viscosity, density, surface tension, evaporation of the formulation ingredients (FI)] of some commercial formulations, and to determine their behaviour in atomization systems. Part II consisted of a field study to use the selected formulation under field conditions to investigate spray deposition, persistence and dissipation patterns of AZ-A in various forestry matrices in a real-world situation.

2.1 Study I—Laboratory evaluation of volatilization and wash-off from treated surfaces, physical properties and formulation behaviour in atomizers

2.1.1 Azadirachtin-A formulations used in the laboratory study

Five nonaqueous formulations were used in the laboratory study. They were:

(i) Margosan-O®, AZ-A 3 g kg^{-1} SL. W.R. Grace and Co., Connecticut, 62 Whitmore Ave., Cambridge, MA 02140, USA.
(ii) Azatin-EC®, AZ-A 30 g kg^{-1} EC, Agridyne Technologies, Inc., 417 Wakara Way, Salt Lake City, Utah 84108, USA.
(iii) Neem-EC®, AZ-A 21 g kg^{-1} EC, Phero Tech Inc., Delta, B.C., Canada.
(iv) RH-9999, AZ-A 200 g kg^{-1} WP, Rohm and Haas Co., Spring House, PA 19477, USA.
(v) Neemix® 4.5, AZ-A 45 g kg^{-1} SL, Grace Biopesticides, W.R. Grace & Co.-Conn. 7379 Route 32, Columbia MD 21044, USA.

2.1.2 Determination of azadirachtin-A loss by volatilization and wash-off from treated surfaces

The experiment was conducted using the commercial formulations without dilution with water, except for the RH-9999. The latter was a wettable powder and therefore was diluted with water to provide an end-use mix containing 35·6 g AI kg^{-1}. To determine the volatilization of AZ-A from treated surfaces, a new method was developed using a glass slide (5 × 2·5 cm) and white spruce [*Picea glauca* (Moench) Voss] foliage (branch tip with fully developed current-year needles).

TABLE 1
Study I—Airborne Concentrations of Azadirachtin-A Following Exposure of Ten Glass Slides and Ten Spruce Branch Tips to a Laminar Wind Flow for 10 Days after Treatment with Five Formulations

Formulation	*Initial concentration (μg per slide or branch)*	*Azadirachtin-A volatilized during 10 days (μg per 10 slides or 10 branches)*[a]	*Final concentration after volatilization (μg per slide or branch)*	*Total mass recovered (% of initial concn)*
		Glass slides		
Margosan-O	25·24	47·34 (18·8)	19·55	96·2
Azatin-EC	319·0	156·5 (4·90)	295·7	97·6
Neem-EC	228·8	137·3 (6·00)	208·8	97·3
RH-9999	372·3	578·5 (15·5)	301·4	96·5
Neemix 4.5	444·6	477·8 (10·8)	387·9	98·0
		Spruce branch tips		
Margosan-O	25·24	67·14 (26·6)	18·03	98·0
Azatin-EC	319·0	462·6 (14·5)	264·8	97·5
Neem-EC	228·8	235·7 (10·3)	197·5	96·6
RH-9999	372·3	443·0 (11·9)	317·2	97·1
Neemix 4.5	444·6	791·4 (17·8)	357·5	98·2

[a] Values in parentheses refer to the percentage of azadirachtin-A volatilized.

Prior to the start of the experiment, the glass slides were coated with the cuticular wax of the spruce branch tips collected from the field [Laird Township (46°22′33″N, 84°01′25″W), about 30 km southeast of Sault Ste. Marie, ON, Canada]. Four branch tips containing current-year growth were placed in chloroform (100 ml) and agitated for 30 s. The branches were removed and discarded. The chloroform solution was flash-evaporated to 40 ml at 37°C. The glass slides (previously weighed) were sprayed with the chloroform–wax solution without allowing it to drip. The slides were allowed to dry and weighed again. This procedure was repeated until the mass reached 0·4022(±0·0197) g (density 0·840 g ml^{-1}, volume 0·4762(±0·0235) ml,), and provided a wax coating of 380 μm thickness on the glass slides.

Droplets of 0·250 μl of each formulation were produced by using a micro-applicator (Instrumentation Specialties Company, 4700 Superior Lincoln, Nebraska 68504, USA) and were applied onto the glass slides at the rate of 40 droplets per slide. Ten replicate slides were treated in the same manner for each formulation. The slides were placed in a tray (35 × 20 cm) in an environmental chamber in darkness at 15°C. The tray was covered with a Perspex® plastic tank (40 × 25 × 60 cm) containing a circular opening (1·2 cm in diameter) on two opposite sides. A piece of Tygon® tubing was mounted through each opening. Air was drawn in through the inlet at the rate of 1·5 litre min^{-1}, allowed to pass over the glass slides and collected at the outlet. The outlet tubing was connected to two air samplers[17,18] (impingers made of Pyrex® glass) containing acetonitrile as the trapping medium, and AZ-A vapour was collected in acetonitrile for 10 days continuously. The impingers were removed every 24 h, and the solvent samples in them were pooled. In this manner, a new set of two impingers containing fresh acetonitrile was used every 24 h. At the end of 10 days, the 10 acetonitrile samples were pooled into five composite samples, i.e. those collected on days 1 and 10 into sample I; days 2 and 9 into sample II; 3 and 8 into sample III; 4 and 7 into sample IV; and 5 and 6 into sample V. These were concentrated by flash evaporation at 30°C prior to analysis of AZ-A using a high-performance liquid chromatographic (HPLC) method reported previously.[19] The final concentrations of AZ-A

on the glass slides after volatilization were also determined by HPLC, although the extracts required cleanup[19] because of the presence of cuticular wax from the foliage. The data are given in Table 1.

Droplets of 250 μl were also applied onto white spruce branch tips containing fully developed current year needles, at the rate of 40 droplets per branch tip. Ten replicate branch tips were treated in the same manner for each formulation. Each branch tip was placed in a Petri dish containing a moist filter paper, and the Petri dishes were arranged in a tray. The tray was placed in the environmental chamber as described above, and volatilization of AZ-A was determined at 15°C. The filter paper was periodically moistened with drops of water to prevent the foliage from drying out during the 10-day exposure period. The data are given in Table 1.

To determine the washoff characteristics of foliar residues, a new method was developed based on the 'worst case scenario.' Since the amount washed off by rain would depend on cumulative rainfall, rainfall intensity, rain-free period etc.,[20] it would not be practical to optimize a formulation for a particular set of parameters for field use, because it would not be possible to predict those parameters ahead of time. Consequently, the need to use 'the worst case scenario' is evident. The details of the method are as follows. Droplets of 0·250 μl were applied on wax-coated glass slides and spruce foliage as described above, and the treated surfaces were set aside for 36 h at 15°C. A 10-ml aliquot of distilled water was taken in a 20-ml beaker and each treated surface (glass slide or branch tip) was rinsed by dipping in the water for 30 s with a gentle swirl. This process was repeated twice for each surface, and the concentrations of AZ-A in the rinse were measured by the HPLC method[19] as above. The residues on the glass slides and spruce foliage remaining after rinsing were also determined. The data are given in Table 2.

2.1.3 Physical properties of the five commercial formulations

Viscosity was measured at temperatures of 5 to 25°C using an Ostwald viscometer.[21] Density was determined using a density bottle. Surface tension was measured using the Fisher Surface Tensiometer, Model 21. The data are given in Table 3.

To determine the evaporation rates of the formulations, an apparatus was constructed by placing a circu-

TABLE 2
Study I—Azadirachtin-A Concentrations Washed off after Rinsing Ten Glass Slides and Ten Spruce Branch Tips in Distilled Water at 36 h after Treatment with Five Formulations

Formulation	*Initial concentration (μg per slide or branch)*	*Azadirachtin-A washed off by rinsing (μg per 10 slides or 10 branches)*[a]	*Final concentration after rinsing (μg per slide or branch)*	*Total mass recovered (% of initial concn)*
		Glass slides		
Margosan-O	25·24	75·22 (29·8)	16·48	95·1
Azatin-EC	319·0	443·4 (13·9)	256·2	94·2
Neem-EC	228·8	223·5 (9·77)	208·8	97·3
RH-9999	372·3	759·5 (20·4)	283·4	96·5
Neemix 4.5	444·6	947·0 (21·3)	341·0	98·0
		Spruce branch tips		
Margosan-O	25·24	81·27 (32·2)	16·25	96·6
Azatin-EC	319·0	631·6 (19·8)	238·3	94·5
Neem-EC	228·8	318·0 (13·9)	192·7	98·1
RH-9999	372·3	737·2 (19·8)	296·3	99·4
Neemix 4.5	444·6	969·2 (21·8)	332·1	96·5

[a] Values in parentheses refer to the percentage of azadirachtin-A washed off by rinsing.

TABLE 3
Study I—Physical Properties of Five Commercial Formulations at Temperatures Ranging from 5 to 25°C

Formulation	*Viscosity* (*mPa s*)	*Density* ($g\ ml^{-1}$)	*Surface tension* ($mN\ m^{-1}$)	*Non-volatile components measured after 7 days* (% *w/w*)
Margosan-O				
5°C	2·492	0·8456	38·87	—
10°C	2·455	0·8428	38·03	—
15°C	2·420	0·8412	37·56	21·55[a]
20°C	2·400	0·8387	37·00	—
25°C	2·360	0·8361	36·65	—
Azatin-EC				
5°C	519·4	1·0669	63·45	—
10°C	335·4	1·0660	62·65	—
15°C	214·3	1·0634	61·77	48·90
20°C	124·9	1·0616	60·88	—
25°C	84·70	1·0586	59·95	—
Neem-EC				
5°C	34·92	1·0924	51·37	—
10°C	29·89	1·0901	50·35	—
15°C	24·45	1·0894	49·66	26·82
20°C	19·68	1·0871	49·02	—
25°C	15·61	1·0867	48·55	—
RH-9999				
5°C	3·601	1·0474	52·07	—
10°C	3·444	1·0469	51·33	—
15°C	2·549	1·0459	50·88	15·16
20°C	2·137	1·0452	50·24	—
25°C	1·866	1·0448	49·87	—
Neemix 4.5				
5°C	23·52	0·9916	37·33	—
10°C	18·04	0·9905	36·78	—
15°C	14·63	0·9879	36·06	47·44
20°C	11·77	0·9859	35·65	—
25°C	9·590	0·9839	35·02	—

[a] Evaporation of formulation ingredients was determined only at 15°C. The values given here represent the percentage of non-volatile components.

lar piece of styrofoam in a Petri dish (3·5 cm in diameter). Four pins (3·5 cm long) were mounted onto the piece of styrofoam to form the apices of a square. A glass microfiber filter (GMFF, 3 cm in diameter) was mounted over the pins. The apparatus was placed on the pan of a Mettler balance with the doors left open, and the balance was placed in an environmental chamber maintained at 15°C. A 200-μl aliquot was pipetted out onto the GMFF, and the initial mass was noted. At different intervals of time, the residual mass of the liquid film on the GMFF was determined and the data were converted into residual mass percent.[22] Figure 1 shows the plot of residual mass percent versus time interval at which the measurement was made. Table 3 presents data on the non-volatile components (w/w%) at 15°C, determined after evaporation for 7 days.

2.1.4 Investigation of formulation behaviour in rotary and pressure atomizers

When a liquid is atomized into droplets, it is subjected to a shearing force. When pressure atomizers are used in still air (e.g., hydraulic nozzles fitted to ground application equipment), the shearing force experienced by the liquid is proportional to the applied pressure. The emerging liquid breaks up into droplets due to its kinetic energy.[23] Since there is no rotational motion of the liquid stream, no centripetal force is involved. In contrast, when a liquid is atomized using rotary atomizers (e.g. the commercial spinning disc atomizers containing fine serrations at the periphery), it is subjected to a centripetal force. At high flow rates, the liquid emerges from the rotating disc in the form of thin streams which then break up into droplets. However, at very low flow rates [such as those used to produce

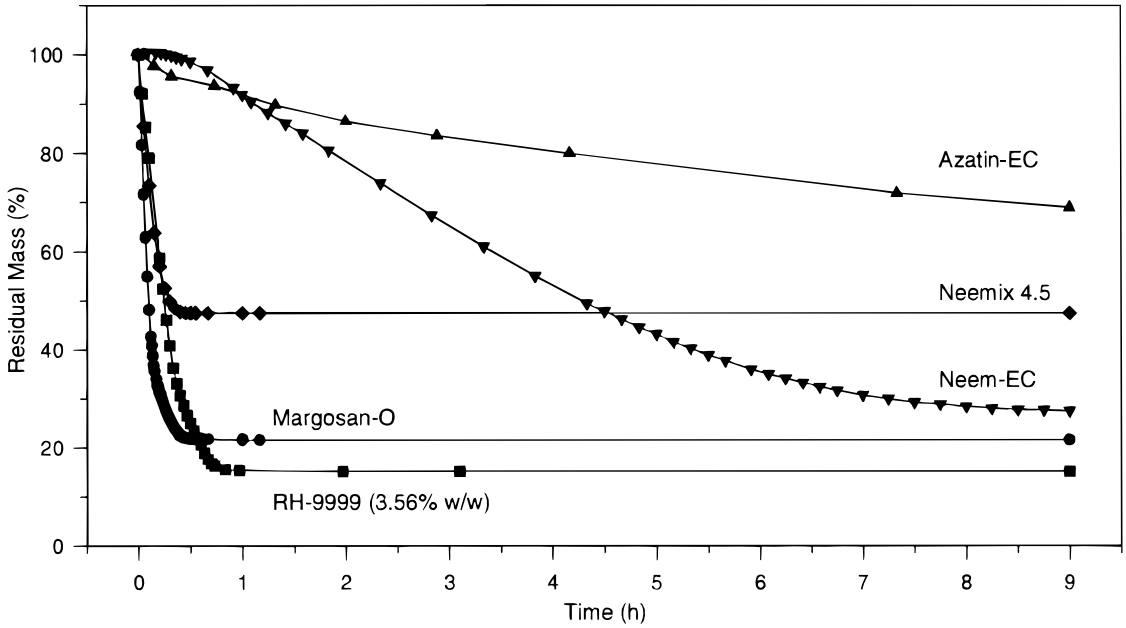

Fig. 1 Evaporation characteristics of five commercial formulations of azadirachtin-A at 15°C.

ultra-low-volume (ULV) droplets with a volume median diameter ($D_{V.5}$) of 50 to 100 μm], the liquid is divided into thin streams which are broken into droplets prior to or during leaving the periphery of the rotating disc.[24] Due to the influence of the centripetal force, the individual components of the droplets could separate into different phases if the intermolecular forces between the ingredients were weak, and if the densities of the ingredients were markedly different. Thus, the probability of phase separation in droplets is higher when liquids are atomized by rotary atomizers than by pressure atomizers.

The procedure was as follows. Aliquots (10 ml) of the formulations were taken in 15-ml graduated centrifuge tubes and maintained at 15°C for 2 h to examine if any phase separation occurred. The observations made are listed in Table 4. Each formulation was then agitated thoroughly, and atomized (at 15°C) in a laboratory chamber (4·3 × 0·9 × 3·05 m) using a hydraulic nozzle (FullJet®, full cone with a strainer, Spraying Systems Co., North Avenue, Wheaton, Illinois, USA), and a spinning disc atomizer (Flak®, Micron Agri-Sprayers, Walkerton, ON, Canada). Spray droplets were collected on glass plates placed at 2·0 m below the atomizer level, and the spots were viewed within 90 s under a microscope for phase separation. The data are given in Table 4.

2.2 Study II—Field evaluation of persistence of azadirachtin-A in forestry matrices, using the formulation selected in Study I

In Study I, Azatin-EC provided minimum volatilization (4·90% of the initial concentration) from glass slides, whereas Neem-EC provided minimum volatilization from spruce foliage (10·3%) (Table 1). On the other hand, the data in Table 2 indicated that Neem-EC caused minimum washoff after rinsing of both surfaces [glass slides (9·77%) and spruce foliage (13·9%)], thus suggesting the advantage of using Neem-EC formulation for field investigation. In the atomization investigation using a rotary atomizer, phase separation did not occur with three (Margosan-O, Neem-EC and Neemix 4.5) of the five formulations (Table 4). Thus, the Neem-EC formulation was considered to be the most appropriate choice for field investigation of AZ-A persistence in different forestry matrices.

2.2.1 Selection of sample trees, litter and soil plots, and placing the glass aquaria for aquatic studies

The study was conducted in Laird Township (46°22′33″N, 84°01′25″W), about 30 km southeast of Sault Ste. Marie). The site consisted of a typical mixed-wood boreal forest area with white spruce trees of varying heights (2 to 6 m). The forest floor was flat and covered with grass and moss patches.

Twelve uniform-sized spruce trees (2·3–2·5 m in height and 7–8 cm in diameter at breast height) were selected randomly from a block to *c.*1·0 ha. The trees were divided into three groups of four. The first group meant for the dosage rate of 40 g AI ha^{-1}, was labelled as A1 to A4. The second group meant for 80 g AI ha^{-1} was labelled as B1 to B4. The last group was labelled as C1 to C4 and served as the control. The ground vegetation under each tree was removed and the area surrounding it was cleared by trimming the interfering foliage or by cutting off the branches from neighbouring trees.

TABLE 4
Study I—Phase Separation in Five Formulations after Standing for 2 h, and in Droplets after Atomization in Pressure and Rotary Atomizers

Parameter measured	*Margosan-O* (*undiluted*)	*Azatin-EC* (*undiluted*)	*Neem-EC* (*undiluted*)	*RH-9999* (*35·6 g kg^{-1}*)[a]	*Neemix 4.5* (*undiluted*)
			Phase separation in formulations after standing for 2 h		
Phase separation	Two phases	Two phases	One phase	Two phases	Two phases
Colour					
Top layer	Light brown	Dark brown	NA[b]	Creamy beige	Brown
Bottom layer	Brownish-green	Brownish-green	NA	White precipitate	Dark brown
			Phase separation in droplet spots on glass plates after atomization in a pressure atomizer		
Minimum diameter of spots (μm)	40	40	35	40	45
Maximum diameter of spots (μm)	450	465	405	315	445
Phase separation in spots	None	None	None	Two phases[c]	None
Colour					
Throughout	Pale brown	Dark brown	Pale brown	NA	Pale brown
Central spot	NA	NA	NA	White	NA
Outer ring	NA	NA	NA	Creamy beige	NA
			Phase separation in droplet spots on glass plates after atomization in a rotary atomizer		
Minimum diameter of spots (μm)	45	30	35	40	35
Maximum diameter of spots (μm)	350	275	315	225	375
Phase separation in spots	None	Two phases[d]	None	Two phases	None
Colour					
Throughout	Pale brown	NA	Pale brown	NA	Pale brown
Central spot	NA	Brownish-green	NA	White	NA
Outer ring	NA	Pale brown	NA	Creamy beige	NA

[a] RH-9999 was a wettable powder, and was mixed with water to provide a suspension containing 35·6 g AI kg^{-1}.
[b] NA = not applicable.
[c] RH-9999 suspension provided a clearly separated central white spot surrounded by an outer ring.
[d] The presence of two phases in droplet spots of Azatin-EC was shown by a clearly separated central spot surrounded by an outer ring.

In the open areas of the block, five litter and five soil plots (two for each of the dosages of 40 and 80 g AI ha^{-1}, and one for control) were selected and the corners were marked by wooden stakes. The area of each plot was about 4 m^2. The litter plots were labelled as L1 and L2 (for the dosage rate of 40 g AI ha^{-1}), and L3 and L4 (for the dosage rate of 80 g AI ha^{-1}). The soil plots were labelled correspondingly as S1 to S4. The control plots used for litter and soil were labelled as CL and CS respectively. Small objects such as fallen branches, twigs, stones etc., were removed from the litter plots and the surface was levelled and packed to the original condition. From the soil plots, however, the overlying litter, moss and organic detritus were also removed to a depth of *c.*10 cm to fully expose the underlying mineral soil to the spray cloud.

For aquatic studies, three rectangular glass aquaria with interior dimensions of 65 × 35 × 28 cm, were used. They were labelled as W1 to W3. Each aquarium was buried in the forest floor to a depth of 20 cm and covered on all sides with earth, to simulate natural lentic systems as closely as possible. Fifty Petri dishes (6 cm diameter, 1·5 cm deep) each filled with 40 g of processed wet sediment[25] [organic matter (OM) 8·4%, sand 21%, silt 44%, clay 35%, CEC 21 meq 100 g^{-1}, surface area 127 m^2 g^{-1}, density (dry wt) 1·20 g ml^{-1}, pH 6·21 (aq. slurry)] collected from a nearby stream, were placed at the bottom of each aquarium. The aquaria were then filled with 40 litres of the stream water (pH 6·32, turbidity 15·3 JTU, specific conductance 11·2 μmhos cm^{-1}, hardness 14·2 mg $CaCO_3$ litre^{-1}) without disturbing the sediment layer.

2.2.2 Application of Neem-EC over trees, litter and soil plots, and fortification of water in the glass aquaria

Prior to spray application, a portable shelter (heavy-duty polyethylene sheets fixed onto a wooden frame) with dimensions of 2·0 × 2·0 × 3·0 m was placed around each tree to prevent any off-target movement of spray droplets into the neighbouring areas. Shelters (2·0 × 2·0 × 1·0 m) were also placed around each litter and soil plot. The enclosures were removed about 30 min after spray application.

Pre-weighed amounts of Neem-EC formulation required for the two dosage levels (40 and 80 g AI in 1·75 and 3·50 litres ha^{-1} respectively), were taken in separate Teflon® bottles and shaken with aliquots of Automate Red B dye (Morton Chemical Ltd, Ajax, ON, Canada) at 10 g kg^{-1}, to provide the corresponding spray mixes for the terrestrial studies. Spray application was made using a hand-held battery-powered Flak® spinning disc atomizer. To apply 40 g AI ha^{-1}, the atomizer settings were so adjusted that one application over the entire surface area of the enclosure would consume 1·75 litres of the formulation (undiluted). To apply the higher dosage rate, the formulation (3·50 litres) was applied in two consecutive applications using the same atomizer settings. Spray application was carried out on 13 June 1995, between 0630 and 0915 h. During application, the average temperature, relative humidity and wind speed were 14·4°C, 80% and 3·9 km h^{-1} respectively. Some meteorological parameters were also monitored during the 16-day sampling period and they are given in Table 5.

TABLE 5
Study II—Maximum and Minimum Temperatures, Average Relative Humidity (RH) and Rainfall During the Field Microcosm Study of Azadirachtin-A

Date June 1995	*Max. temp. (°C)*	*Min. temp. (°C)*	*Average RH (%)*	*Rainfall (mm)*
13	23·4	5·3	80	—
14	23·1	5·1	77	—
15	26·9	8·7	79	—
16	27·1	11·9	81	—
17	27·8	13·1	80	—
18	28·4	13·6	81	—
19	28·7	14·9	79	—
20	28·2	12·1	79	—
21	27·1	12·9	82	—
22	28·4	11·7	78	—
23	27·9	12·2	82	—
24	28·6	14·7	80	—
25	28·9	16·9	76	2·6
26	24·4	17·6	79	19·4
27	25·2	18·6	82	1·0
28	21·5	18·1	84	0·4

To fortify the glass aquaria with the Neem-EC formulation, pre-weighed aliquots without the dye were mixed with 50 ml of the stream water to provide dosages ten times higher (i.e., 400 and 800 g AI ha^{-1}) than those used in the terrestrial plots. These mixes were used to fortify the water in aquaria W1 and W2 respectively. Each mix was added dropwise, using a pipet to cover the entire area of the water surface. After fortification, the contents were allowed to equilibrate for 0·5 h before the initial sampling was done. After each sampling interval, the water level in each aquarium was marked with a waterproof pen, and just prior to the next sampling, this water level was restored by topping-up with fresh stream water to counteract any loss by evaporation.

2.2.3 Assessment of droplet size spectra and spray mass deposit on artificial samplers

Several types of artificial sampler were used in the field investigation. One type was prepared by mounting one Kromekote® card (K-card, 10 × 10 cm; Intercity Papers Ltd, Mississauga, ON, Canada) on one aluminium sheet (12 × 12 cm) and two glass plates (each 5·0 × 7·5 cm) joined together by a masking tape on another aluminium sheet. The two aluminium sheets were joined together by a masking tape. These samplers were placed on the ground, one at each of the four corners of the litter and soil plots. Another type of sampler was prepared by mounting one K-card only on an aluminium sheet, and four of these were placed on 1·5-m-high wooden stakes in the four corners of each tree enclosure. For deposit measurements on spruce trees, the GMFF papers were cut into thirty 1 × 50 mm strips, and these were attached to an aluminium wire to simulate spruce branch tips. These samplers, described as GMFF clusters, were mounted, one on each of the four quadrants of a sample tree, at mid-crown level. All samplers were placed in the field 15 to 30 min before spray application, and were collected at 30 to 45 min post-spray. The K-cards were wrapped in aluminium foil and stored in a desiccator. The deposits on the glass plates and GMFF clusters were extracted with acetonitrile and the extracts were collected in amber-coloured glass bottles. The bottles were placed in coolers containing ice-packs and brought to the residue laboratory where they were stored at − 20°C until analysis.

2.2.4 Sampling of terrestrial and aquatic matrices

Samples of spruce needles (one-year-old, 1994 growth) and shoots (1995 growth) were collected at 2·0 h before spray application (pre-spray) and at 0·5, 3, 9, 27, 33, 52, 76, 98, 144 and 192 h post-spray. Four branches (20 cm long) were collected from the mid-crown level of each tree (one branch from each quadrant), packed in plastic bags, and brought to the laboratory in coolers containing ice packs. In the laboratory, each group of 16

TABLE 6
Study II—Degradation of Azadirachtin-A in Spruce Needles,[a] Shoots[a] and Bark[a] after Application of Neem-EC Formulation at Two Dosage Rates

	Dosage 40 g AI ha^{-1}			*Dosage 80 g AI ha^{-1}*		
Time after application (h)	*Needles (ng g^{-1}) (±SD)*	*Shoots (ng g^{-1}) (±SD)*	*Bark (ng g^{-1}) (±SD)*	*Needles (ng g^{-1}) (±SD)*	*Shoots (ng g^{-1}) (±SD)*	*Bark (ng g^{-1}) (±SD)*
0·5	2771 (±306)	1241 (±141)	589 (±61)	5630 (±706)	2587 (±303)	1346 (±149)
3	2199 (±208)	1006 (±96)	566 (±74)	4798 (±663)	2114 (±270)	1269 (±130)
9	1786 (±191)	873 (±76)	501 (±59)	3665 (±404)	1742 (±168)	1167 (±121)
27	1372 (±144)	499 (±56)	471 (±41)	3069 (±286)	1065 (±117)	996 (±112)
33	986 (±100)	313 (±34)	499 (±48)	2771 (±198)	683 (±59)	907 (±106)
52	463 (±54)	171 (±21)	384 (±41)	1881 (±186)	341 (±60)	799 (±91)
76	266 (±33)	ND[b]	226 (±36)	789 (±91)	174 (±25)	647 (±89)
98	105 (±14)	ND	219 (±31)	349 (±48)	ND	501 (±74)
144	ND	ND	140 (±24)	186 (±24)	ND	381 (±49)
192	ND	ND	113 (±19)	ND	ND	276 (±41)

[a] Average pre-spray moisture content: needles, 46·9 (±4·3)% ($n = 12$); shoots, 59·7 (±5·0)% ($n = 12$); and bark, 34·5 (±3·3)% ($n = 10$).
[b] ND = not detected; LOD and LOQ: needles, shoots and bark, 50 and 100 ng g^{-1}, respectively.

branch tips was pooled randomly into four composite samples. The one-year-old needles were separated from shoots using clean scissors, wrapped in aluminium foil, put into plastic bags and stored at −20°C until extracted and analyzed. The bark of the branch tips was also removed carefully using a sharp knife, mixed well and stored as above.

Litter samples (OM > 80%, sand 24·2%, silt 36·4%, clay 39·4%, pH 5·0) from the four treatment plots (two plots per dosage) and from the control plot were sampled at the same time intervals as the foliage. Four cores were taken (two from each of the two plots sprayed at the same dosage) per sampling period, by driving a stainless steel tube (4·2 cm diameter and 25 cm long) into the ground to a depth of 2·5 cm. The cores were removed using a clean plunger and the four cores belonging to the same dosage were pooled into two composite samples, packed in coolers and brought to the laboratory. The stainless steel tube was cleaned with acetone between samplings. In the laboratory, each pooled sample was macerated with a Hobart® chopper (Hobart Manufacturing Co. Ltd, Don Mills, ON, Canada) after the stones, twigs, roots etc. were removed. Samples were sieved (2-mm opening) and stored at −20°C until analysis.

Soil samples (OM 3·5%, sand 18·1%, silt 41·2%, clay 40·7%, pH 5·3) were collected, using a stainless steel auger (2·0 cm diameter, 40 cm long), at the same sampling intervals as the litter and foliage. Soil cores were taken randomly at each sampling period to a depth of 2·5 cm, except for the three sampling intervals, 9, 33 and 76 h post-spray, during which 7·5-cm-deep cores were taken. The latter cores were sliced with a clean knife into three segments, corresponding to 0–2·5 cm, 2·5–5·0 cm and 5·0–7·5 cm, to study the vertical mobility of AZ-A through the soil profile. The auger was cleaned with acetone between samplings. Twelve soil cores (six from each of two plots) were taken per dosage at each sampling interval. The samples were randomly pooled to form three composite samples for triplicate measurements. All samples were sieved (2 mm opening) after removing stones, debris, roots, etc., wrapped in aluminium foil, put into labelled plastic bags, placed in coolers, brought to the laboratory and stored at −20°C until analysis. The control samples were collected and stored similarly.

Water and sediment samples from the control and treated aquaria were taken 2·0 h before treatment (pre-treatment) and at 13 post-treatment intervals corresponding to 0·5, 3, 9, 27, 33, 52, 76, 98, 144, 192, 240, 312 and 384 h after application. At each sampling interval, 100 ml of water was pipetted out (in triplicate) from each aquarium from 5-cm depth by dipping a pipet and drawing up the liquid using the Brinkmann Pipet Helper® (Brinkmann Instruments Ltd, Mississauga, ON, Canada). The tip of the pipet was moved around the subsurface while sampling, to cover as much area as possible, to achieve sample homogeneity. The samples were transferred into Teflon® bottles, placed in coolers and brought to the laboratory for storage at −20°C. Sediment samples were collected at the same sampling frequency as water. The lid of each Petri dish was gently lowered to cover the Petri dish containing the sediment, by using long-handled stainless steel tongs with wide jaws. The covered Petri dish was carefully lifted out of the aquarium without disturbing the packed sediment and the aquarium water. Each Petri dish was wiped dry, wrapped in aluminium foil, put in a plastic bag, brought to the laboratory and stored at −20°C. The control samples were collected and stored similarly.

TABLE 7
Study II—Degradation of Azadirachtin-A in Clay Loam Forest Soil[a] and Litter[a] after Application of Neem-EC Formulation at Two Dosage Rates

	Dosage 40 g AI ha^{-1}		*Dosage 80 g AI ha^{-1}*	
Time after application (h)	*Soil (ng g^{-1}) (±SD)*	*Litter (ng g^{-1}) (±SD)*	*Soil (ng g^{-1}) (±SD)*	*Litter (ng g^{-1}) (±SD)*
0·5	78 (±7)	368 (±29)	155 (±13)	689 (±47)
3	66 (±9)	277 (±23)	113 (±14)	578 (±52)
9	47 (±6)	189 (±21)	98 (±8)	446 (±33)
27	12 (±3)	98 (±11)	84 (±9)	301 (±27)
33	7 (±2)	48 (±7)	74 (±4)	257 (±22)
52	4 (±2)	13 (±3)	44 (±6)	132 (±16)
76	ND[b]	ND	17 (±3)	77 (±13)
98	ND	ND	7 (±2)	51 (±7)
144	ND	ND	ND	23 (±6)
192	ND	ND	ND	ND

[a] Average pre-spray moisture content: soil, 16·7 (±1·7)% ($n = 6$); litter, 28·7 (±4·7)% ($n = 8$).
[b] ND = not detected; LOD and LOQ: soil, 2 and 4 ng g^{-1} respectively; litter, 5 and 10 ng g^{-1}, respectively.

2.2.5 Extraction and analysis

Analytical grade AZ-A (purity >95%) was purchased from Sigma Chemical (St. Louis, Missouri, USA). All samples were extracted and analyzed for AZ-A residues using the HPLC method reported previously.[19] Briefly, all the solid matrices were processed[25] and extracted with aqueous methanol, concentrated, partitioned with hexane and re-extracted with dichloromethane. After evaporation of the dichloromethane, the residues were dissolved in ethyl acetate, cleaned on a Florisil® mini-column, eluted with ethyl acetate, and analyzed using a reversed-phase C-18 column, with UV detection at 210 nm and acetonitrile-water gradient system. Analysis of AZ-A in the stream water required only the steps from dichloromethane extraction onwards. Glass plate and GMFF extracts were flash-evaporated to dryness, the residues taken up in ethyl acetate and analyzed without any column cleanup. The limits of detection (LOD) and limits of quantification (LOQ) were respectively: water 1 and 2 ng ml^{-1}; sediment 1 and

TABLE 8
Study II—Azadirachtin-A Concentrations in Stream Water and Sediment[a] in an Aquatic Model System Kept Under Field Conditions

	Dosage 400 g AI ha^{-1}		*Dosage 800 g AI ha^{-1}*	
Time after application (h)	*Water (ng ml^{-1}) (±SD)*	*Sediment (ng g^{-1}) (±SD)*	*Water (ng ml^{-1}) (±SD)*	*Sediment (ng g^{-1}) (±SD)*
0·5	218·9 (±4·1)	ND[b]	406·8 (±7·1)	ND
3	194·6 (±7·6)	3 (±1)	375·5 (±3·9)	7 (±1)
9	159·5 (±7·0)	5 (±1)	334·5 (±8·0)	15 (±3)
27	115·8 (±9·1)	7 (±2)	274·0 (±6·2)	18 (±3)
33	113·1 (±8·9)	4 (±1)	229·4 (±4·1)	13 (±2)
52	82·3 (±6·3)	2 (±1)	172·7 (±3·1)	5 (±1)
76	44·4 (±4·4)	ND	85·5 (±2·8)	2 (±1)
98	23·5 (±3·9)	ND	42·7 (±3·3)	ND
144	8·5 (±0·9)	ND	19·0 (±1·7)	ND
192	4·3 (±1·2)	ND	12·1 (±1·2)	ND
240	ND	ND	6·9 (±0·7)	ND
312	ND	ND	3·1 (±0·3)	ND
384	ND	ND	ND	ND

[a] Average moisture content of sediment = 42·0 (±4·5)% ($n = 20$).
[b] ND = not detected; LOD and LOQ: water, 1·0 and 2·0 ng ml^{-1}, respectively; sediment, 1·0 and 2·0 ng g^{-1}.

TABLE 9
Study II—Droplet Spectra on Kromekote Cards, and Spray Deposit of Azadirachtin-A on Glass Microfiber Filter (GMFF) Clusters and Glass Plates, after Application of Neem-EC Formulation at Dosage Rates of 40 and 80 g AI ha^{-1} over Spruce Trees, Litter and Soil Plots

	40 g AI in 1·75 litres ha^{-1}			*80 g AI in 3·50 litres ha^{-1}*		
Parameter	*Tree encl.*	*Litter*	*Soil*	*Tree encl.*	*Litter*	*Soil*
Droplet size spectra on Kromekote cards[p]:						
$D_{N.5}$ (μm)	49	61	60	70	55	62
$D_{V.5}$ (μm)	56	65	70	81	67	77
D_{max} (μm)	100	110	110	125	130	110
D_{min} (μm)	11	11	11	7	5	11
Droplet density	106	72	74	122	162	106
Spray mass deposit on samplers[p,q]:						
GMFF	208	—	—	449	—	—
Glass plate	—	22·8	25·6	—	53·5	50·3
Recovery	—	0·570	0·640	—	0·669	0·629

[p] Values refer to the mean of total number of samplers used for each dosage rate.
[q] The deposit data on GMFF clusters are expressed in ng AI cm^{-2}. The data on glass plates are expressed in g AI ha^{-1}. The recovery fraction was calculated as the g AI ha^{-1} deposited divided by the g AI ha^{-1} applied.

2 ng g^{-1}; litter 5 and 10 ng g^{-1}; soil 2 and 4 ng g^{-1}; and spruce needles, shoots and bark 50 and 100 ng g^{-1}. Mean recovery levels of the analyte, studied by fortifying the pre-spray and control samples of litter, soil, spruce needles, shoots and bark each at 0·05 to 1·0 μg g^{-1}, sediment at 0·01 to 0·50 μg g^{-1}, and water at 1·0 to 50 ng ml^{-1}, ranged from 82 to 104% with coefficients of variation from 6 to 11%. Residue data recorded in Tables 6 to 8 include the correction for recovery efficiency. External standards were interspersed among the samples to check consistency of HPLC response. In addition, quality control programs

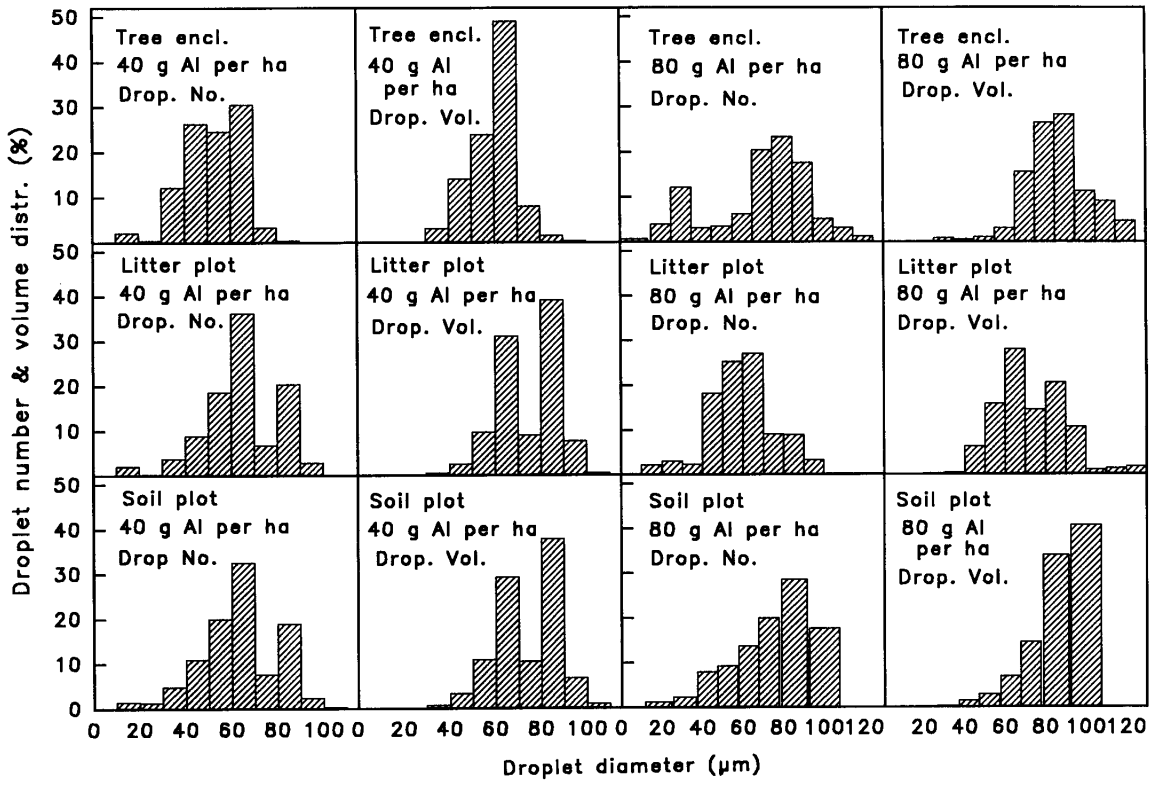

Fig. 2 Droplet number and volume distribution percentages according to size category on Kromekote cards after application of Neem-EC using a rotary atomizer.

were introduced throughout the course of AZ-A analysis to monitor the accuracy and precision of the method used.

None of the pre-spray or control samples contained any AZ-A and there was no evidence of co-extracted materials causing interference with the identification and quantification of the chemical. To study the storage stability of AZ-A, processed pre-spray and control samples of the different matrices were fortified with varying levels of the analyte and stored at $-20°C$ for 60 days. At intervals of time, aliquots were taken and analyzed using the HPLC method. The recoveries were quantitative for a 16-day period for water and 30-day period for the other substrates. Beyond these time intervals, a gradual decrease in recovery with time was noted, ranging from *c.*15% on day 45 to 30% on day 60 for solid matrices, and from 30% on day 30 to 65% on day 50 for water. Therefore, all the samples were analyzed within the time frame reported for quantitative recovery.

The moisture contents of sediment, litter, soil, spruce needles, shoots and bark samples were determined according to the AOAC method,[26] by taking 2·0- to 3·0-g aliquots of the processed substrates ($n = 3$) and drying them in a thermostatic oven at 120°C until constant weights were obtained.

2.2.6 Analysis of droplet stains on Kromekote cards

The droplet stains on the 16 mid-crown K-cards (four K-cards per tree, four trees per dosage), and the eight cards from each of the litter and soil plots (four cards per plot, two plots per dosage) were analyzed using an American Optical Microscope at magnifications of 25× and 40×. The LOD was 20 μm for the droplet stains on the cards. The data were recorded automatically (Wild Leitz MM 235 Measuring Device, Wild Leitz Canada Ltd, Willowdale, ON, Canada). Spread factor data were generated as described by Sundaram *et al.*[27] and the stain diameters were converted into the corresponding spherical diameters. The data were used to compute the number and volume median diameters ($D_{N.5}$ and $D_{V.5}$ respectively), maximum and minimum diameters (D_{max} and D_{min} respectively) and the results are recorded in Table 9, together with droplet density (droplets cm^{-2}). The droplet number and volume distribution percentages according to different size category are also presented as histograms in Fig. 2.

2.2.7 Spray mass deposit on glass microfiber filter (GMFF) clusters and on glass plates

The deposits on AZ-A obtained on the 16 GMFF clusters (four clusters per tree, four trees per dosage) were analyzed by the HPLC method[19] and the data were converted into ng AI cm^{-2} using the surface area of the clusters. Similarly, the amount of AZ-A deposits on glass plates corresponding to the two litter and soil plots were converted into g AI ha^{-1}. The results are given in Table 9.

3 RESULTS AND DISCUSSION

3.1 Study I—Laboratory evaluation of azadirachtin-A loss from treated surfaces, physical properties, and formulation behaviour in pressure and rotary atomizers

3.1.1 Volatilization and washoff of azadirachtin-A from treated surfaces

Table 1 presents data on the amount of AZ-A volatilized during the 10-day exposure to a laminar wind flow in the environmental chamber at 15°C. The amount lost varied with the type of surface, being consistently higher from the spruce foliage than from the glass plates. For example, the percentage loss from glass plates ranged from 4·90 for Azatin-EC to 18·8 for Margosan-O, whereas the corresponding values from spruce foliage were 14·5 and 26·6% respectively. The data thus indicate appreciable differences between the two surfaces. It appears that AZ-A required more energy for volatilization from the non-living waxy layer on the glass plate than from the live spruce foliage.

There were also appreciable differences in volatilization of AZ-A among the five formulations (Table 1). For example, Azatin-EC and Neem-EC formulations provided the lowest values for percentage loss (4·90 and 6·00% respectively) from glass plates. However, Neem-EC and RH-9999 formulations provided the lowest amount lost (10·3 and 11·9% respectively) from spruce foliage. In contrast, Margosan-O provided the highest values (18·8% from glass plates and 26·6% from spruce foliage). The data thus indicate the role of formulation ingredients on volatilization of AZ-A.

The total mass recovered, i.e. the amount lost by volatilization plus the amount left on the treated surfaces, was close to 100% for all formulations (Table 1), regardless of the type of surface used. The data suggest that during the 10-day exposure period, AZ-A did not undergo appreciable metabolic and/or degradative changes under the experimental conditions that existed in the environmental chamber.

Table 2 presents data on the amount of AZ-A lost by wash-off after rinsing the treated surfaces in distilled water. Again, the amount lost was consistently higher from the spruce foliage than from the glass plates. For example, the percentage loss from glass plates ranged from 9·77 for Neem-EC to 29·8 for Margosan-O, whereas the corresponding values for spruce foliage ranged from 13·9 to 32·2%. Similarly to volatilization, AZ-A seemed to require more energy for wash-off from the non-living waxy layer on the glass plate than from the live spruce foliage.

There were also appreciable differences in the wash-off of AZ-A among the five formulations (Table 2). For example, Neem-EC and Azatin-EC formulations pro-

vided the lowest values for percentage loss (9·77 and 13·9%) from glass plates, whereas the corresponding values from spruce foliage were higher 13·9 and 19·8%, respectively. However, Margosan-O provided the highest values (29·8% from glass plates and 32·2% from spruce foliage). The data thus indicate the role of formulation ingredients on wash-off of AZ-A. Furthermore, the total mass recovered, i.e. the amount washed off plus the residual amount left on the treated surfaces, was close to 100% for all formulations (Table 2), regardless of the type of surface used. The data thus indicate that AZ-A did not seem to undergo appreciable degradative changes during the 36-h storage prior to rinsing.

The criterion used for selecting a formulation for field testing was based on maximum retention of AZ-A after volatilization and wash-off from spruce foliage, since our intention was to increase the foliar stability of AZ-A. Our present data indicated that Neem-EC provided the least amount of volatilization and wash-off from foliage. Therefore, the formulation of choice for field studies, based on these factors, appeared to be Neem-EC, although the findings on atomization studies should also be taken into account.

3.1.2 Physical properties of the five commercial formulations

Table 3 gives the viscosity, density and surface tension values of the five formulations, and Fig. 1 provides the evaporation characteristics. Among the four properties, viscosity varied the most, and density varied the least. For example, Azatin-EC showed the highest viscosities, ranging from 84·7 to 519·4 mPa s when the temperature decreased from 25 to 5°C. In contrast, Margosan-O provided the lowest values, ranging from 2·360 to 2·492 mPa s. Neem-EC showed the highest density values, and Margosan-O the lowest. The surface tensions of Azatin-EC were the highest, ranging from 59·95 mN m^{-1} at 25°C to 63·45 mN m^{-1} at 5°C, whereas the corresponding values for Neemix 4.5 were the lowest, ranging from 35·02 to 37·33 mN m^{-1}. Regarding the evaporation of formulation ingredients, the aqueous formulation of the wettable powder RH-9999 evaporated the most because of the lowest percentage of non-volatile components (15·16% measured after 7 days), whereas Azatin-EC evaporated the least and provided the highest amount of non-volatile components (48·90%) (Table 3). The importance of physical properties of the end-use formulations lies in the selection of atomization parameters, *viz.* flow rate, nozzle orifice, application pressure, speed of the rotary atomizer etc. However, physical properties may play a role in phase separation in droplets either during or after atomization, resulting in nonuniform distribution of the AI in spray droplets. Consequently, the influence of physical properties on phase separation in droplets after atomization was also considered as one of the parameters for formulation selection for field testing.

3.1.3 Phase separation in droplets after atomization in pressure and rotary atomizers

Table 4 presents findings on phase separation in droplets (determined by microscopic examination) after atomization in a hydraulic nozzle (full cone) and in a rotary atomizer (Flak). For the sake of comparison, the findings on phase separation in 10-ml aliquots of formulations after standing for 2 h are also listed in Table 4. The results indicate that only Neem-EC provided one phase, while the rest separated into two phases. Phase separation occurred with two formulations (Azatin-EC and RH-9999) in droplets after atomization in a rotary atomizer, whereas only one formulation (RH-9999) showed phase separation after atomization in a pressure nozzle. The high viscosity of Azatin-EC would cause a decrease in the rotational speed of the atomizer, and this, in combination with density differences in the two phases, could have resulted in phase separation in the droplets. The colour of the phases in droplets was similar to that observed in formulations after 2 h standing. For example, Azatin-EC provided a dark brown layer at the top and a brownish-green layer at the bottom, in the 10-ml samples set aside for 2 h. These colours corresponded to those observed in the outer ring and central spot respectively, in droplets collected on glass plates after atomization in Flak. Similar behaviour was also noted in the RH-9999 droplets.

Based on phase separation in droplets deposited on glass plates, three formulations, *viz.* Margosan-O, Neem-EC and Neemix 4.5, were considered to have the potential for providing uniform distribution of AI in droplets after atomization in a rotary atomizer (Flak was chosen to apply the formulation under field conditions). Nonetheless, for maintaining homogeneity of phases in droplets, with minimum volatilization and washoff of residues from foliage, Neem-EC was considered to be the most appropriate choice for field testing. Consequently, Neem-EC was selected for field studies to investigate the persistence of AZ-A in terrestrial and aquatic matrices in a real-world situation.

3.2 Study II—Field evaluation of persistence of azadirachtin-A in forestry matrices, using the formulation selected in Study I

3.2.1 Initial residues and persistence of azadirachtin-A in spruce needles, shoots and bark samples

The residues of AZ-A (ng g^{-1}, fresh weight) in one-year-old spruce needles, shoots and bark samples after application of Neem-EC at the two dosage rates (40 and 80 g AI ha^{-1}) are given in Table 6. Each value represents the mean ($\pm$SD) obtained from analysis of four replicate samples (individual samples were pooled selectively to

obtain four composite samples). To minimize the variability in results, all residues were measured on a dry weight basis, and the data were adjusted to the constant pre-spray moisture level of each matrix (needles 46·9%, shoots 59·7% and bark 34·5%).

The mean initial residue levels in the needles, shoots and bark showed marked differences regardless of the dosage rate applied. The needles appeared to have collected spray droplets more efficiently than the shoots, probably because of their open geometry. The shoots, being somewhat more dense and compact than needles, received much less deposit. The bark samples received the lowest amount of AZ-A, partly because they were shielded from the spray droplets by the canopy foliage.

The initial residues declined from all substrates with time; however, the rate of decline was relatively rapid in shoots, moderate in needles and slow in bark at both dosage levels. For example, at 27 h post-spray, about 40·5, 52·5 and 77% (average of the values at the two dosage rates) of the initial amounts remained in shoots, needles and bark respectively. At 52 h post-spray, the corresponding average values were 13·5% of AZ-A in shoots, 25% in needles and 62% in bark. No detectable levels of residues were found in shoots sampled at 76 h at 40 g AI ha^{-1}. However, at 80 g AI ha^{-1} a time period of 98 h was required to reach the level below the LOD. In general, the duration of persistence was longer in one-year-old needles than in shoots (Table 6). Persistence was the longest in bark, since residues were still measurable until the last sampling period (192 h post-spray).

The dissipation kinetics of AZ-A in the three plant matrices were calculated using first-order equations. The equations, coefficients of determination (R^2), rate constant 'C' and DT_{50} (the time required for dissipation of 50% of the initial deposit) values are given in Table 10 for the different substrates. The DT_{50} values at both dosages decreased in the order of bark > needles > shoots, whereas the rate constants increased in the order of bark < needles < shoots. The average DT_{50} values were respectively: 73·9 h (bark) 27·25 h (needles) and 18·5 h (shoots). The similarity in the DT_{50} values at the two dosages, indicates that the rate of loss of AZ-A from the three substrates was not influenced by the initial concentrations.

The relatively high DT_{50} values for AZ-A residues in bark could be due to reduced enzymatic activity in the matrix, coupled with sheltering of the active material from sunlight by the canopy foliage. In contrast, the rapid loss of the chemical in shoots could be due to high enzymatic activity,[28] and also due to dilution during the rapid growth of shoots after the bud flush. Comparatively, the slower rate of loss of AZ-A from needles could be due to lower enzymatic activity coupled with negligible growth dilution.[29] Loss of AZ-A due to rainfall did not occur, because there was no precipitation during the study period (i.e. 14–20 June 1995) (Table 5). AZ-A is a systematic insecticide,[30] and how much of the deposited AZ-A had translocated to the untreated parts of the conifer trees is not known, and requires further investigation.

3.2.2 Initial residues and persistence of azadirachtin-A in litter and soil samples

The residues of AZ-A in forest litter and soil are given in Table 7. The results, as for foliage, were measured on a dry weight basis, but were adjusted to the constant pre-spray moisture level (litter 28·7% and soil 16·7%). The mean initial concentrations (ng g^{-1} wet weight) in the litter plots at the 40 and 80 g AI ha^{-1} dosage levels were 368 and 689 respectively. These residues were on average 4·5 times higher than the corresponding values found in the soil plots (78 and 155 ng g^{-1} respectively). The relatively high deposition on the litter surface could

TABLE 10

Study II—Regression Equation, Rate Constant (C), DT_{50} and R^2 for the Dissipation of Azadirachtin-A from Aquatic and Terrestrial Matrices Sampled During a Field Microcosm Study

Matrix	*Dosage* (*g AI* ha^{-1})	*Regression equation*	*Rate constant*[a] *C*	DT_{50} (*h*)[a]	*Coeff. detr.*[a] (R^2)
Water	400	$Y = 208{\cdot}5\ e^{-0{\cdot}0203\ t}$	0·0203	34·1	0·993
	800	$Y = 406{\cdot}8\ e^{-0{\cdot}0184\ t}$	0·0184	37·7	0·993
Soil	40	$Y = 79{\cdot}48\ e^{-0{\cdot}0650\ t}$	0·0650	10·7	0·998
	80	$Y = 137{\cdot}8\ e^{-0{\cdot}0231\ t}$	0·0231	30·0	0·959
Litter	40	$Y = 349{\cdot}8\ e^{-0{\cdot}0591\ t}$	0·0591	11·7	0·990
	80	$Y = 647{\cdot}4\ e^{-0{\cdot}0296\ t}$	0·0296	23·4	0·990
Spruce needles	40	$Y = 2574\ e^{-0{\cdot}0297\ t}$	0·0297	23·3	0·983
	80	$Y = 5246\ e^{-0{\cdot}0222\ t}$	0·0222	31·2	0·978
Spruce shoots	40	$Y = 1205\ e^{-0{\cdot}0381\ t}$	0·0381	18·2	0·991
	80	$Y = 2486\ e^{-0{\cdot}0368\ t}$	0·0368	18·8	0·994
Spruce bark	40	$Y = 583{\cdot}8\ e^{-0{\cdot}0097\ t}$	0·0097	71·6	0·976
	80	$Y = 1292\ e^{-0{\cdot}0091\ t}$	0·0091	76·2	0·991

[a] Values of C, DT_{50} and R^2 were calculated after logarithmic transformation of the regression equation.

be due to the porous nature of the matrix (composed primarily of decayed plant material mixed with small amounts of mineral constituents), which acted as a good collector for the spray droplets. The initial residues decreased exponentially with time in both substrates. At 27 and 52 h post-spray, the residues in litter at 40 g AI ha^{-1} amounted to 26·6 and 3·5% of the initial values respectively, but at 80 g AI ha^{-1}, the corresponding values were 43·7 and 19·2% (Table 7). At the same time intervals the residues in soil amounted to 15·4 and 5·1% respectively at the lower dosage, but 54·2 and 28·4% at the higher dosage.

The data given in Table 10 indicate that at the 40 g AI ha^{-1} dosage, the DT_{50} value was 11·7 h for the litter and 10·7 h for the soil samples, thus indicating similar rate of AZ-A loss from the two substrates. However, at the 80 g AI ha^{-1} dosage, the corresponding values were 23·4 h for the litter and 30·0 h for the soil, indicating that forest litter did not act as a micro-sink for AZ-A, contrary to the observations made earlier on other lipophilic pesticides.[29,31] Microbial action and photolysis could be the major contributing factors in the dissipation of AZ-A, in addition to the roles played by other physicochemical factors such as volatilization, co-distillation etc.[32]

3.2.3 Initial residues and persistence of azadirachtin-A in stream water and sediment

The residues of AZ-A in water and sediment samples collected at different intervals of time are given in Table 8. To minimize the variability in results, the residues in sediment were measured on a dry weight basis, but the data were adjusted to the average moisture content (42%) of the substrate. The initial residue levels in water varied according to the dosage applied. At 400 g AI ha^{-1}, the mean initial concentration was 218·9 ng ml^{-1}, whereas at the 800 g AI ha^{-1} dosage, the corresponding value was 406·8 ng ml^{-1}. In contrast, the initial sediment samples did not contain any detectable residues (LOD 1·0 ng g^{-1}). A gradual build-up of AZ-A in sediments began at 3 h after fortification, and the mean residues at 3 h were 3 and 7 ng g^{-1} at the 400 and 800 g AI ha^{-1} dosages respectively.

AZ-A concentrations in water decreased gradually with time. At 33 h post-treatment, the residue levels were 113 and 229 ng ml^{-1} respectively at the two dosages, and these corresponded to 51·7 and 56·4% of the initial values. However, at 192 h post-treatment, the residues were only 2·0 and 3·0% of the initial levels. At 240 h after fortification, no residues were found in the aquarium treated at 400 g AI ha^{-1}, but measurable amounts of residues (6·9 ng ml^{-1}) still remained in the aquarium treated at 800 g AI ha^{-1}. The dissipation mechanisms include hydrolysis,[33] oxidation,[34] photolysis,[35] microbial action[33] and volatilization. No vapour pressure data for AZ-A are reported in literature to evaluate its volatilization potential. However, AZ-A is a complex molecule with C-C π-bonds, ester linkages and epoxide rings.[9] Based on structural considerations and the types of intermolecular forces involved, volatilization (or sublimation) and co-distillation from water are quite likely. Our own data recorded in Table 1 (Study I) support the volatilization hypothesis, although the data were obtained using glass plates and spruce foliage and not using a water surface.

Unlike the decline of initial residues in water with time, the AZ-A residues in sediment increased with time during initial stages, reached peak amounts of 7 and 18 ng g^{-1} at 27 h after treatment at the two dosages respectively, and declined gradually afterwards. No residues were found after 52 and 76 h in water treated at the 400 and 800 g AI ha^{-1} dosage rates respectively. The average sediment concentration factor (concentration in sediment/concentration in water) at the peak level was very low (*c.*0.065), in spite of the high OM (8·4%), silt (44%) and clay (35%) contents, and large surface area (127 m^2 g^{-1}), all of which were favourable for adsorption of hydrophobic insecticides to sediment. This clearly demonstrates that bottom sediments in water bodies, like ponds, lakes and streams, in a forest environment could seldom act as efficient sinks for AZ-A. Our earlier studies with other lipophilic pesticides indicated that adsorption to sediment was an efficient pathway to remove the active material from water bodies.[36] Azadirachtin-A behaves like a hydrophilic pesticide, favouring its retention in water rather than partitioning into sediment, and the little that is adsorbed (if any) is probably desorbed readily back into water. The processes that contributed to decline of residues from sediment would include (in addition to desorption) microbial action, hydrolysis and to a lesser extent, photolysis due to exposure to diffused light passing through the water column.

3.3 Droplet size spectra and spray deposits of azadirachtin-A on artificial samplers

Table 9 gives the spray deposits of AZ-A (in ng AI cm^{-2}) on the GMFF clusters placed at the mid-crown level of trees, and on glass plates in g AI ha^{-1} placed at the forest floor, after application of Neem-EC at the two dosages of 40 and 80 g AI ha^{-1}. Average deposit levels on the GMFF clusters increased with increasing dosage rate. A similar trend was also observed on the glass plates at the forest floor level. On average, the amount of AZ-A deposited was higher at 80 g AI ha^{-1} than at 40 g AI ha^{-1}. However, the percentage deposition was similar at both dosage rates. This observation is in agreement with our studies conducted recently using the hormonal insecticide, tebufenozide.[29]

Similarly to the spray mass deposit on samplers, the droplet density values also showed an increase with dosage. This is attributable to the volume rate of 3·50

litre ha^{-1} used at the high dosage rate compared to the 1·75 litre ha^{-1} used at the low dosage rate. In addition, a slight increase in droplet size parameters ($D_{N.5}$ and $D_{V.5}$) was observed on the K-cards placed in the tree enclosures and on the soil plots, at the high dosage rate. For example, the $D_{N.5}$ and $D_{V.5}$ on K-cards in the tree enclosure were 49 and 56 μm respectively at the lower dosage rate, whereas they were 70 and 81 μm respectively at the higher dosage rate. Similarly, the $D_{N.5}$ and $D_{V.5}$ values on cards placed on the soil plots were 60 and 70 μm respectively at the lower dosage rate, but 62 and 77 μm at the higher dosage rate. However, the K-cards on the litter plots showed similar $D_{N.5}$ and $D_{V.5}$ at the two dosage rates, but yet the droplet density and percentage recovery values were higher at the 80 g AI ha^{-1} than at the 40 g AI ha^{-1} (Table 9). The reason for this is not clear, and requires further investigation.

4 CONCLUSIONS

The present investigation indicated that, under laboratory conditions, azadirachtin-A volatilized during exposure to a laminar wind flow, and volatilization occurred more from spruce foliage than from wax-coated glass plates. The amount volatilized also depended on the formulation, because AZ-A volatilized the least (10.3%) from spruce foliage when Neem-EC was applied, and the most (26·6%) when Margosan-O was applied. Similarly to volatilization, the loss due to wash-off by rinsing was consistently higher from spruce foliage than from the glass plates. Again, Neem-EC provided the lowest wash-off (13·9%) from spruce foliage, whereas Margosan-O provided the highest (32·2%).

The data on physical properties indicated that viscosity varied the most, and density varied the least. Except for the very high viscosity of Azatin-EC, the physical properties of the remaining four formulations were in agreement with the values reported previously for emulsion concentrates (ECs).[37] The findings on phase separation in droplets after atomization in a pressure atomizer indicated that only the wettable powder formulation, RH-9999, provided two phases in droplets collected on glass plates. However, when atomized using a rotary atomizer, Azatin-EC and RH-9999 provided two phases in droplets. Based on the potential for maximum retention of AZ-A on spruce foliage after volatilization and wash-off, and on the minimum tendency for phase separation in droplets after atomization, Neem-EC was considered to be the most appropriate formulation for field testing to investigate environmental fate and persistence of AZ-A in forestry substrates.

The field microcosm study conducted after application of Neem-EC at two dosage rates (40 and 80 g AI ha^{-1}) indicated that the duration of persistence of AZ-A depended on the dosage rate applied. The chemical persisted for 3 to 6 days in one-year-old spruce needles and young shoots, whereas it persisted at measurable amounts beyond 8 days in spruce bark. Persistence in litter and soil also varied with the dosage, and the duration ranged from 3 to 6 days. Persistence in stream water and sediment placed in glass aquaria in the field showed that AZ-A rapidly disappeared from both matrices, in spite of the higher dosage rates applied (400 and 800 g AI ha^{-1}). The duration of persistence ranged from 8 to 13 days in water, but only from 2 to 3 days in sediment. The residual concentrations in the substrates at different intervals of time after treatment were fitted into first-order kinetics equations. The DT_{50} values ranged from 10·7 h (for soil) to 71·6 h (for bark) at the lower dosage rate, and from 18·8 h (for shoots) to 76·2 h (for bark) at the higher dosage rate (Table 10). Environmental persistence of a pesticide depends on its physicochemical properties, its concentration in the forestry substrates, the type of formulation, method of application, and a multitude of environmental factors (i.e. substrate type, temperature, moisture, pH, rainfall, wind flow, sunlight, micro-organisms etc.). Considering the complexity of the mechanisms involved, it is difficult to predict the life-time of AZ-A in a specific substrate. However, the intrinsic properties of the chemical and the extrinsic factors encountered in this field microcosm study indicate that AZ-A is appreciably labile and short-lived in different forestry matrices, with low DT_{50} values.

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